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Attorney Docket No.: 5636.210-US

PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Nielsen et al.

Confirmation No: 6089

Serial No.: 10/038,723

Group Art Unit: 1652

Filed: January 2, 2002

Examiner: To be assigned

For: Glucoamylase Variants

## SUPPLEMENTAL PRELIMINARY AMENDMENT

Commissioner for Patents Washington, DC 20231

Sir

Before examination, please amend the above-identified application as follows:

## IN THE CLAIMS:

Please cancel claims 29-140 without prejudice or disslaimer.

## Please add new claims 141-202, as follows:

141. An isolated variant of a parent glucoamylase comprising a mutation at one or more of the following positions in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in a homologous glucoamylase having at least 80% homology with the amino acid sequence shown in SEQ ID NO:2:

1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 10, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 28, 29, 31, 32, 33, 34, 35, 40, 41, 42, 43, 44, 45, 46, 47, 49, 51, 53, 56, 58, 60, 61, 62, 73, 74, 75, 76, 77, 78, 79, 80, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 109, 110, 111, 113, 114, 115, 117, 118, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 234, 235, 237, 238, 239, 240, 242, 243, 244, 245, 287, 288, 289, 290, 291, 292, 294, 295, 296, 298, 299, 300, 301, 303, 304, 308, 311, 314, 315, 316, 318, 319, 334, 335, 336, 337, 338, 339, 340, 341, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 384, 388, 390, 394, 397, 398, 399, 402, 403, 404, 405, 406, 409, 412, 413, 414, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 461, 462, 463, 464, 465, 466, 467, 488, 469, 470.

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- 142. The variant of claim 141, wh rein said h mologous glucoamylase has at least 90% homology to SEQ ID NO:2.
- 143. The variant of claim 141, wherein said homologous glucoamylase has at least 95% homology to SEQ ID NO:2.
- 144. The variant of claim 141, wherein said variant comprises a mutation at position 1 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 145. The variant of claim 141, wherein said variant comprises a mutation at position 2 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 146. The variant of claim 141, wherein said variant comprises a mutation at position 3 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 147. The variant of claim 141, wherein said variant comprises a mutation at position 4 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoarnviase.
- 148. The variant of claim 141, wherein said variant comprises a mutation at position 5 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 149. The variant of claim 141, wherein said variant comprises a mutation at position 6 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous discoamviase.
- 150. The varian' of claim 141, wherein said variant comprises a mutation at position 7 in the amino acid sequence shown in SEQ iD NO:2 or at a corresponding position in said homologous glucoarnylase.



- 151. The variant of claim 141, wherein said variant comprises a mutation at position 8 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 152. The variant of claim 141, wherein said variant comprises a mutation at position 9 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 11 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamviase.
- The variant of claim 141, wherein said variant comprises a mutation at position 10 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous gluccamviase.
- 155. The variant of claim 141, wherein said variant comprises a mutation at position 12 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- 156. The variant of claim 141, wherein said variant comprises a mutation at position 13 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 14 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 15 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- 159. The variant of claim 141, wherein said variant comprises a mutation at position 16 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 160. The variant of claim 141, wherein said variant comprises a mutation at position 17 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 161. The variant of claim 141, wherein said variant comprises a mutation at position 18 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 162. The variant of claim 141, wherein said variant comprises a mutation at position 19 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 163. The variant of claim 141, wherein said variant comprises a mutation at position 21 in the aming acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 164. The variant of claim 141, wherein said variant comprises a mutation at position 22 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 165. The variant of claim 141, wherein said variant comprises a mutation at position 23 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 166. The variant of claim 141, wherein said variant comprises a mutation at position 24 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamviase.

- 167. The variant of claim 141, wherein said variant comprises a mutation at position 25 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 168. The variant of claim 141, wherein said variant comprises a mutation at position 26 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 28 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 170. The variant of claim 141, wherein said variant comprises a mutation at position 29 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous diucoamviase.
- 171. The variant of claim 141, wherein said variant comprises a mutation at position 31 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 172. The variant of claim 141, wherein said variant comprises a mutation at position 32 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous alucoamviase.
- The variant of claim 141, wherein said variant comprises a mutation at position 33 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 34 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- 175. The variant of claim 141, wherein said variant comprises a mutation at position 35 in the amino acid s quence shown in SEQ ID NO:2 or at a corresponding position in said homologous olucoamviase.
- 176. The variant of claim 141, wherein said variant comprises a mutation at position 40 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 177. The variant of claim 141, wherein said variant comprises a mutation at position 41 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 178. The variant of claim 141, wherein said variant comprises a mutation at position 42 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 179. The variant of claim 141, wherein said variant comprises a mutation at position 43 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 180. The variant of claim 141, wherein said variant comprises a mutation at position 44 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 181. The variant of claim 141, wherein said variant comprises a mutation at position 45 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 182. The variant of claim 141, wherein said variant comprises a mutation at position 46 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- 183. The variant of claim 141, wherein said variant comprises a mutation at position 47 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous qiucoamvlase.
- 184. The variant of claim 141, wherein sald variant comprises a mutation at position 49 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- 185. The variant of claim 141, wherein said variant comprises a mutation at position 51 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 186. The variant of claim 141, wherein said variant comprises a mutation at position 53 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 187. The variant of claim 141, wherein said variant comprises a mutation at position 56 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 188. The variant of claim 141, wherein said variant comprises a mutation at position 58 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 189. The variant of claim 141, wherein said variant comprises a mutation at position 60 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 190. The variant of claim 141, wherein said variant comprises a mutation at position 61 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- 191. The variant of claim 141, wherein said variant comprises a mutation at position 62 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- 192. The variant of claim 141, wherein said variant comprises a mutation at position 73 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 193. The variant of claim 141, wherein said variant comprises a mutation at position 74 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 194. The variant of claim 141, wherein said variant comprises a mutation at position 75 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 195. The variant of claim 141, wherein said variant comprises a mutation at position 76 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 196. The variant of claim 141, wherein said variant comprises a mutation at position 77 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- 197. The variant of claim 141, wherein said variant comprises a mutation at position 78 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 198. The variant of claim 141, wherein said variant comprises a mutation at position 79 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous alucoamviase.

- 199. The variant of claim 141, wherein said variant comprises a mutation at position 80 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 200. The variant of claim 141, wherein said variant comprises a mutation at position 93 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 201. The variant of claim 141, wherein said variant comprises a mutation at position 94 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous giucoamylase.
- 202. The variant of claim 141, wherein said variant comprises a mutation at position 95 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous gluccamylase.
- 203. The variant of claim 141, wherein said variant comprises a mutation at position 96 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- 204. The variant of claim 141, wherein said variant comprises a mutation at position 97 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 98 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- The variant of claim 141, wherein said variant comprises a mutation at position 99 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

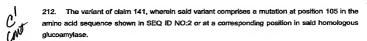


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207. The variant of claim 141, wherein said variant comprises a mutati n at position 100 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

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- The variant of claim 141, wherein said variant comprises a mutation at position 101 in the aming acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.
- The variant of claim 141, wherein said variant comprises a mutation at position 102 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 210. The variant of claim 141, wherein said variant comprises a mutation at position 103 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 211. The variant of claim 141, wherein said variant comprises a mutation at position 104 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- The variant of claim 141, wherein said variant comprises a mutation at position 106 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 214. The variant of claim 141, wherein said variant comprises a mutation at position 107 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamvlase.



- 216. The variant of claim 141, wherein said variant comprises a mutation at position 110 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 217. The variant of claim 141, wherein said variant comprises a mutation at position 111 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 218. The variant of claim 141, wherein said variant comprises a mutation at position 113 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 219. The variant of claim 141, wherein said variant comprises a mutation at position 114 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 220. The variant of claim 141, wherein said variant comprises a mutation at position 115 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 221. The variant of claim 141, wherein said variant comprises a mutation at position 117 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoarnylase.
- 222. The variant of claim 141, wherein said variant comprises a mutation at position 118 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoarnylase.



- 223. The variant of claim 141, wherein said variant comprises a mutation at position 200 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 224. The variant of claim 141, wherein said variant comprises a mutation at position 201 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 225. The variant of claim 141, wherein said variant comprises a mutation at position 202 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 226. The variant of claim 141, wherein said variant comprises a mutation at position 203 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 227. The variant of claim 141, wherein said variant comprises a mutation at position 204 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 228. The variant of claim 141, wherein said variant comprises a mutation at position 205 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 229. The variant of claim 141, wherein said variant comprises a mutation at position 206 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 230. The variant of claim 141, wherein said variant comprises a mutation at position 207 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.



- 231. The variant of claim 141, wherein said variant comprises a mutation at position 208 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 232. The variant of claim 141, wherein said variant comprises a mutation at position 209 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 233. The variant of claim 141, wherein said variant comprises a mutation at position 210 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 234. The variant of claim 141, wherein said variant comprises a mutation at position 211 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 235. The variant of claim 141, wherein said variant comprises a mutation at position 234 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
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- 236. The variant of claim 141, wherein said variant comprises a mutation at position 235 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 237. The variant of claim 141, wherein said variant comprises a mutation at position 237 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.
- 238. The variant of claim 141, wherein said variant comprises a mutation at position 238 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoarrylase.

240. The variant of claim 141, wherein sald variant comprises a mutation at position 240 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoarrylase.

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292. The variant of claim 141, wherein said variant comprises a mutation at position 242 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

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2)4. The variant of claim 141, wherein said variant comprises a mutation at position 243 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

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2/94. The variant of claim 141, wherein said variant comprises a mutation at position 244 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

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246. The variant of claim 141, wherein said variant comprises a mutation at position 245 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucqamylase.

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7.248. The variant of claim 141, wherein said variant comprises a mutation at position 287 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

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2/17. The variant of claim 141, wherein said variant comprises a mutation at position 288 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous diucoamylase.

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2/48. The variant of claim 141, wherein said variant comprises a mutation at position 289 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

279. The variant of claim 141, wherein said variant comprises a mutation at position 290 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

250. The variant of claim 141, wherein said variant comprises a mutation at position 291 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous clucoamylase.

251. The variant of claim 141, wherein said variant comprises a mutation at position 292 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

252. The variant of claim 141, wherein said variant comprises a mutation at position 294 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamylase.

The variant of claim 141, wherein said variant comprises a mutation at position 295 in the amino acid sequence shown in SEQ ID NO:2 or at a corresponding position in said homologous glucoamytase.

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